United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine

Soil and Growing Medium Sampling Protocol
Revised April 22, 2008

See http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/ for latest approved protocol.

Soil and Growing Media Sampling:

- Infested soil or growing media will look exactly the same as un-infested soil or growing media. Therefore all soil and media must be handled carefully. All tools used to collect soil or media samples must be disinfected with 10% bleach solution, quaternary ammonium solution or flame-sterilized with a propane torch between blocks. All soil and organic material should be removed from the tools prior to disinfection. Care should also be taken not to transfer soil or growing media from one block to the next on shoes or clothing. All sampling equipment should be cleaned and disinfected prior to entering a new nursery block. Care must be taken to ensure that un-infested soil or growing media is not contaminated by infested soil or growing media. If the areas of soil/media infestation are known or suspected sample these quarantine block and work toward the destruction block(s).

Preparing for sampling:

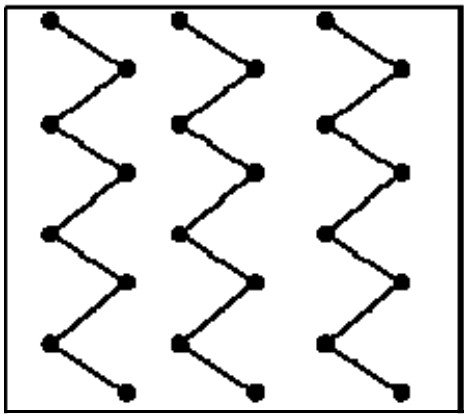
- Soil and growing media samples should be collected as composite samples. Composite samples of growing media should be kept separate from soil samples. A composite sample consists of a mixture of sub-samples. Sub-samples (See Figure 1) are small amounts of soil (or media) removed from the ground (or pot) and added together to form a composite sample. The use of sub-sampling increases the chances of finding *P. ramorum* if it is present. Samples should contain a maximum of 500-ml (volume) of soil and/or growing media (1/2 of a quart-size Ziploc bag). The number of composite samples collected will depend upon the size of the nursery block being sampled (see Table 1). There should be at least two samples, one for growing media and one for soil, unless all plants and associated growing media were destroyed or the plants are not on soil (e.g. on concrete or asphalt). If the surface of soil is covered with gravel take sub-samples from the soil beneath the gravel. If water permeable weed block is present, either covered with gravel or under gravel, the weed block should be removed prior to soil sampling.

Table 1: Number of composite samples collected based on nursery block size.

Size of Treated Site (acres)	Sq Ft	No. of Soil and Growing Media Samples Collected (total)
0.00< n < 0.25	n <10,890	5 (10)
0.25 < n < 0.5	10,890 <n < 21,780	10 (20)
0.50 < n < 1.0	21,780 <n < 43,560	20 (40)
n >1.0	n > 43,560	30 (60)

- Each composite sample will consist of at least five sub-samples collected from soil or growing media within the targeted area. While five is a minimum, it is preferable to take 24 sub-samples of soil or growing media for each sample, provided the area is large enough (for soil samples) and enough plants are present (for growing media samples). Sub-samples should be collected according the pattern in the diagram below (Figure 1). Alternatively, if fallen leaves or other debris from the infected plants are present; sub-sampling may be targeted towards those areas. The location of each composite sample should be maintained (preferably by GPS but at least by flagging) in case follow-up treatment of the soil or growing media for *P. ramorum* is required. Composite samples may also be collected from neighboring blocks of un-infested plants using the same steps. If you are collecting from blocks of un-infested plants, collect the composite soil/growing media samples from these blocks first to minimize the risk of contaminating un-infested soil/growing media. If all potentially-infested growing media has been destroyed with the infected plants, collect composite samples from the remaining host plants within 2- to 10-m of the originally infected plants that have been placed on hold. Preferentially target the growing media of those plants that are down slope (e.g., based on watering patterns) of the originally infected plants.

Figure 1: Recommended pattern for collection of sub-samples for composite soil and/or growing media samples.



Soil Baiting

It is possible to follow the below procedure and not successfully bait and culture *P. ramorum.* This may be due to *P. ramorum* not being present, but may be due to dormancy of *P. ramorum*. To address this dormancy potential and to better enable the diagnostician to detect *P. ramorum* when present, mix the soil well and split the soil samples when they arrive in the laboratory. Once the samples are well mixed and split, place one of the split sample halves into cold storage at approximately 4 degrees C for one month. Bring samples out from cold room after one month has passed, leave samples at room temperature for two days and repeat soil baiting process This baiting can be done in conjunction with the final baiting required fore the quarantine release survey. The samples should be processed as shown below.

To prepare soil bait, briefly soak the pears (select unripe green pears) or Rhododendron leaves in a mild detergent solution to remove any pesticide residues. Rinse the baits well and drain.

Leaving the soil in the Ziploc bag, add enough sterile deionized water to saturate and cover soil with about 2.5 cm (1") of water. Do not mix the soil and water.

Use two pears or leaves per soil sample. With a black sharpie pen, label one side of the pears or leaves with the soil sample number and date processed. The USDA Forest Service recommends the following bait selection criteria in *Stream Baiting Protocol: 2007 National Phytophthora ramorum Early Detection Survey of Forests*, issued March 20, 2007. See http://fhm.fs.fed.us/sp/sod/sod.shtm for latest approved protocol.

Bait Selection

- Use leaves from a population of native or naturalized rhododendrons, if possible. The population should be sufficiently large to supply needed leaves for the survey duration.

- Variation in Pr susceptibility among rhododendron species/cultivars in laboratory inoculation has been published, but field and lab studies have shown that leaves of common native and naturalized species perform acceptably as Pr bait.

- Leaf size can vary considerably among species and cultivars. If bait leaves are quite small (8 cm x 3 cm at the widest point or smaller), use 2 leaves in each pocket of the bait bag.

- If the source of leaves is nursery-grown or naturalized landscape plants, ensure that they have been free of fungicides and other pesticides for a minimum of 6 weeks before using as bait.

- Source plants should be mostly free of dieback and leaf symptoms. Use 1 year-old leaves as free as possible from leaf symptoms (spots, blight, chlorosis), insect damage, and mechanical damage. Do not use newly formed, succulent leaves. Leaves formed in the present year may be used after full leaf expansion and a period of hardening in summer.
- Bait leaves wrapped in paper towels moistened with chlorinated tap or sterile water and sealed in a plastic bag may be stored refrigerated for up to 1 week before use. Do not use well water or stream water for stored leaves.

Carefully push each pear or leaf into the wet soil and water until the bait is immersed halfway. Leave the labeled side of the bait out of the water. Seal the Ziploc bag and leave bait in the soil/water mixture for at least 48- hr at room temperature.

After 48-hr, remove the baits and wash off any clinging soil into Ziploc bag. Set the bait on a moistened paper towel in a sealed container at room temperature for 7-d to let any potential disease symptoms develop. The soil/water mixture must be autoclaved before disposal.

Examine the bait daily for developing symptoms. Pears infected with *P. ramorum* will display lesions that are round, brown, somewhat leathery in texture, with undefined edges. Colorless, watery, and/or soft lesions are generally caused by other pathogens (especially *Pythium* spp.).

Rhododendron leaves that have become infected with *P. ramorum* will exhibit 'diffuse' leaf spots usually with the midvein most affected.

Under the laminar flow hood, cut eight to 10 pieces of pear or leaf from the edge of the developing lesion or leaf spot and insert into the PARP medium. Write the sample number and date processed on the underside of the Petri dish. Seal the dish with parafilm and incubate and treat as described in the USDA approved *Guidelines for Isolation by Culture and Morphological Identification of Phytophthora ramorum* at: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml